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0021-972X/84/5805-0868$02.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 58, No. 5
Printed in U.S.A.

In Vitro Immunoreactivity to Propylthiouracil, Methimazole, and Carbimazole in Patients with Graves' Disease: A Possible Cause of Antithyroid Drug-Induced Agranulocytosis*

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ABSTRACT. Studies of *in vitro* immunoreactivity to propylthiouracil (PTU), methimazole (MMI), and carbimazole (CARB), as assessed by peripheral blood lymphocyte transformation and 2 antibody tests, were carried out in 12 patients with Graves' hyperthyroidism who had developed agranulocytosis during treatment with PTU (11 patients) or CARB (1 patient) from 1 week to 10 yr earlier. Significant lymphocyte transformation responses to antithyroid drugs (stimulation indices > mean ± 2 SD for normal subjects) were found in 5 of 6 patients tested, in 1 patient to PTU only, in 3 patients to MMI only, and in 1 patient to both PTU and MMI, but in none of 10 patients currently being treated with PTU who did not develop agranulocytosis. Circulating antibodies causing neutrophil agglutination in the presence of antithyroid drugs were demonstrated, using the indirect Coombs test, in 5 of 7 patients tested, in 2 patients to PTU only, in 3 patients to CARB only and in 1 patient (the only one tested with MMI) to PTU and MMI. Lymphocyte transformation and antibody tests to PTU were both carried out in 6 patients. Of these, both tests were positive in one patient, both negative in 3 patients, and 1 negative and 1 positive in 2 patients. In the 1 patient in whom both tests were carried out with CARB (patient 3), tests were negative, whereas in the 1 patient in whom both tests were carried out with MMI (patient 3), 1 test was positive, whereas the other was negative. Thus, in patients in whom both tests were carried out using the same drug, correlation between lymphocyte transformation responses and the detection of neutrophil antibodies was found in 5 of 6 cases. Antibodies reactive with neutrophils were also detected in 2 of the 5 patients tested using an enzyme-linked immunosorbent assay. In this test antibodies to PTU or MMI were not demonstrated.

Possible mechanisms for the neutrophil depression in relation to these findings are discussed. It is concluded that patients with Graves' disease may be prone to develop this complication of antithyroid drug therapy because of underlying immunological abnormalities. (*J Clin Endocrinol Metab* 58: 868, 1984)

AGRANULOCYTOSIS is an uncommon but well recognized complication of antithyroid drug therapy. Although the mechanism is unknown, there are several features that suggest hypersensitivity including the suddenness and unpredictable time of onset, which, however, is always more than about 10 days after the commencement of the drugs, and occasional association with rashes, eosinophilia, fever, vasculitis and lupus-like illnesses (1–6).

Agranulocytosis is most common with propylthiouracil (PTU). With renewed interest in PTU as an antithyroid drug because of its additional action of blocking the peripheral conversion of T_4 to T_3 (7, 8) and a possible immunosuppressive role (9–11), it is clearly important to determine the mechanism of the neutrophil depression in order to predict those patients likely to develop this complication.

In this study, tests of peripheral blood lymphocyte (PBL) transformation in response to PTU, methimazole (MMI), and carbimazole (CARB) were carried out on patients with Graves' disease who developed agranulocytosis while being treated with PTU or CARB. As controls, lymphocyte transformation tests were carried out in patients with Graves' disease currently being treated with PTU, who had no adverse reactions. In addition, tests for circulating antibodies against neutrophils and antithyroid drugs were performed using two techniques.

Subjects and Methods

Twelve patients with Graves' hyperthyroidism, all women, aged 17–35 (mean age, 27 yr), who developed agranulocytosis

Received October 25, 1983.

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* This work was supported by Grants AM-07917 and AM-16497 from the NIADDK, NIH, and by Grant MA-6982 from the Medical Research Council of Canada.

within 2 weeks to 1¼ yr after commencing PTU (11 patients) or CARB (1 patient) were studied 1 week to 10 yr after development of the blood dyscrasia. In all, the illness was associated with sudden onset of fever, sore throat, and cervical lymphadenopathy. Recovery occurred within 2 weeks after discontinuation of the drug in all patients. All but 1 of the patients were euthyroid at the time of testing, having been treated with radioactive iodine (8 patients) or thyroidectomy (3 patients) after recovery from the blood dyscrasia, from 2–10 yr earlier. The exception was a patient (patient 5, Table 1) tested soon after development of the agranulocytosis while still hyperthyroid. Only 1 patient (patient 4) had a history of other drug allergies (to penicillin) and no patient had evidence of other autoimmune disorders. No patient was taking other potentially bone-marrow suppressive drugs at the time of agranulocytosis. All but 1 of the patients were treated with standard doses of antithyroid drugs. The exception was the patient who developed agranulocytosis while being treated with CARB (patient 3, Table 1). At the time of onset of the blood dyscrasia she was receiving 200 mg CARB/day.

Also studied were 1) 15 euthyroid patients with Graves' disease currently being treated (for 3–18 months) with PTU without side effects, 9 women and 6 men, aged 30–72 (mean age, 54 yr); 2) 3 women aged 34–64 (mean age, 54 yr) with toxic nodular goiter currently being treated with PTU without side effect; and 3) 21 normal subjects, 17 women and 4 men aged 21–68 (mean age, 42 yr).

PBL were harvested using an Isopaque-Ficoll gradient (12) and cultured in medium TC 199 (Difco Laboratories, Detroit, MI) supplemented with 10% autologous serum, 10% fetal calf serum, 200 mM glutamine, and 50 μg/ml of both penicillin and streptomycin, for 6 days at 37 C in a humidified atmosphere of 95% air and 5% CO_2 in the presence of PTU, MMI, or CARB, each at a concentration of 10 μg/ml. Quadruplicate cultures, each containing 2×10^6 lymphocytes in 1.2 ml medium, were set up. Lymphocyte transformation was assessed by adding 1.2 μCi [^{3}H]thymidine (SA, 5 Ci/mmol, New England Nuclear, Boston, MA) 18 h before termination of the cultures. At the time of harvesting, supernatants were discarded and the cell proteins precipitated in 10% trichloracetic acid, washed, dried in methanol, and the radioactivity measured in Bray's solution. Results were expressed as stimulation indices (SI), *i.e.* ratio of counts per min/2×10^6 lymphocytes in stimulated cultures to counts per min/2×10^6 lymphocytes in control (unstimulated) cultures and a positive test as an SI more than the upper limit of normal (mean + 2 SD). Lymphocyte transformation tests were carried out in 6 patients.

Circulating autoantibodies against PTU, MMI, and CARB were tested for using a modified antihuman globulin (Coombs) test. In this test patient or normal serum was incubated with or without antithyroid drugs together with neutrophils from either the patient (patient 3 only) or a normal subject and agglutination of neutrophils assessed when antihuman globulin was added. Neutrophils were obtained as the buffy coat layer from 20 ml citrated anticoagulated blood. Only neutrophils which were not agglutinated by Coombs reagent (indicating the presence of surface Fc receptors) were used in the test. Briefly, neutrophils, together with serum from patients with agranulocytosis or normal subjects, and antithyroid drugs (all 50 μg/ml) were mixed gently and incubated for 1 h at 37 C in a water bath. The cells were washed three times in saline and antihuman γ-globulin (Coombs reagent) added to a 50-μl aliquot. This was centrifuged for 10 min and the neutrophils examined microscopically for agglutination. This was assessed as positive (+) or negative (−). In no case was borderline or doubtful agglutination observed. In all cases appropriate controls, namely, (1) neutrophils and patient's serum without antithyroid drugs; (2) neutrophils and antithyroid drugs without patient's serum; (3) neutrophils, antithyroid drugs, and normal serum; and (4) neutrophils, patient's serum, and unrelated drug, were included. Coombs tests were done in seven patients. Antibodies against neutrophils and antithyroid drugs were also tested for using an enzyme-linked immunosorbent assay (ELISA) in which normal neutrophils (10^5 per well), PTU, or MMI (each 0.1, 1, and 10 μg/ml) or both neutrophils and antithyroid drug, were coated onto the surface of microtiter wells. Patient serum (diluted 1:100) was incubated with antigen overnight at 4 C, followed by incubation with antihuman immunoglobulin G conjugated to β-galactosidase for 3 h at 37 C. Controls included tests with no antigen and tests with unrelated drugs. Results were expressed as optical density (od) at 410 nm. A positive test was taken as an od more than the upper limit of normal (mean + 2 SD).

Statistical analyses

Differences between patient and control groups were evaluated using the Students' *t* test.

Results

The results of lymphocyte transformation tests are summarized in Fig. 1. Tests were carried out in 6 of the 12 patients. Quadruplicate values were always within 20% of one another. For normal subjects SI ranged from 0.02–2.4, with a mean of 1.2 [±0.15 (SE)], n = 14) in tests with PTU, and from 0.03–2.2 with a mean of 1.23 (±0.20, n = 11) in tests with MMI. The upper limit of normal was 2.2 for PTU and 2.6 for MMI. Tests were positive to PTU in 2 patients (patients 3 and 4) with antithyroid drug-induced agranulocytosis, and to MMI in 4 patients (patients 1, 2, 4, 6), whereas the mean values (3.67 ± 1.83, n = 6 and 10.1 ± 5.1, n = 6, respectively) were both significantly greater than those for normal subjects (*t* tests, $P < 0.01$ and $P < 0.001$, respectively). In all patients with positive tests mean (±SE) counts per min with antithyroid drug was significantly greater (*t* tests, $P < 0.05$) than that for tests without drug. Cell viability, assessed by trypan blue exclusion, was always more than 90% at 3 days of culture and more than 75% at 6 days, and not altered by MMI or PTU. Tests were positive to MMI alone in 3 patients (patients 1, 2, 6), to PTU alone in one patient (patient 3), and to both drugs in 1 patient (patient 4) (Table 1). The patient whose PBL responded to PTU alone (patient 3) was the patient who developed

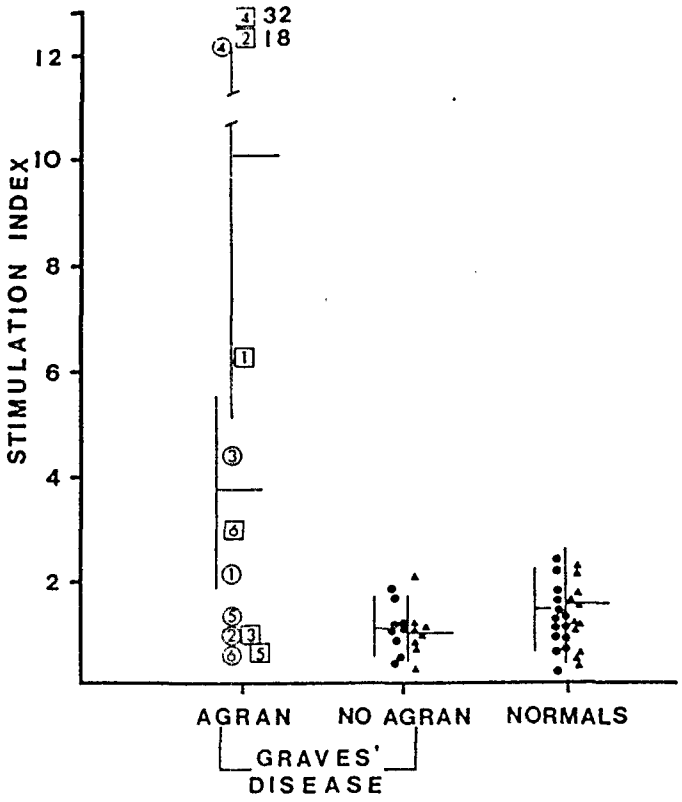

FIG. 1. Peripheral blood lymphocyte transformation in response to PTU and MMI in 1) patients with Graves' disease with antithyroid drug-induced agranulocytosis (AGRAN), 2) patients currently being treated with PTU without reaction, and 3) normal subjects. Results are expressed as SI. *Horizontal bars*, mean values (±SE). ○, PTU; □, MMI for patients with agranulocytosis; the numbers representing individuals with agranulocytosis, corresponding to those in Table 1. ●, PTU; ▲, MMI for patients not having a drug reaction, and normal subjects.

agranulocytosis while receiving CARB, to which she did not respond *in vitro* (SI 1.1, not shown). Positive tests were not found in any of 10 patients with hyperthyroid Graves' disease currently being treated with PTU who did not develop agranulocytosis and the mean (±SE) SI for this group (1.05 ± 0.47, n = 9, and 0.99 ± 0.49, n = 8, for tests with PTU and MMI, respectively) were not significantly different from those for normal subjects (P = NS).

Circulating antibodies against neutrophils were detected using the indirect Coombs test in 5 of the 7 patients tested (Table 1). Positive tests were found only in the presence of the patients' serum and antithyroid drug, irrespective of whether the neutrophils were obtained from patient (patient 3 only) or from a normal subject. Tests were positive with PTU alone in 1 patient (patient 2), with CARB alone in three patients (patients 1, 6, and 7), and with PTU and MMI in the 1 patient in whom the test was carried out with MMI, namely the patient with CARB-induced agranulocytosis (patient 3). Tests with serum from 4 patients currently treated with PTU who did not develop agranulocytosis, from 3 patients with toxic adenoma currently being treated with PTU, and from 5 normal subjects were negative with all three drugs in all (data not shown).

Finally, using an ELISA, antibodies reactive with normal neutrophils, not influenced by the addition of antithyroid drugs, were detected in 2 of the 5 patients tested (patients 8 and 9), but in none of 5 patients currently treated with PTU who did not develop agranulocytosis (Fig. 2), although the mean od for the group (0.21 ± 0.08, n = 5) was not significantly greater than that for normal subjects (0.11 ± 0.02, n = 7, P = NS). Tests were negative to PTU or MMI alone in all 5 patients. Coombs tests and lymphocyte transformation tests were not done in these 5 patients.

Discussion

Drug-induced blood dyscrasias are caused by a variety of mechanisms, including direct toxic effects and immunological reactions. Although immunologically-mediated

TABLE 1. Clinical details and summary of PBL transformation and antibody tests in seven patients with antithyroid drug-induced agranulocytosis

Patient no.	Age at testing	Sex	Antithyroid drug treatment	Sensitization time[a]	Time (at testing) since agranulocytosis[b]	Positive PBL transformation			Neutrophil agglutination[c]		
						PTU	MMI	CARB	PTU	MMI	CARB
1	35	F	PTU	3 mo	10 yr	−	+	NT	−	NT	+
2	25	F	PTU	3 mo	3 yr	−	+	NT	+	NT	−
3	32	F	CARB	3 mo	4 yr	+	−	−	+	+	−
4	20	F	PTU	5 mo	3 yr	+	+	NT	−	NT	−
5	17	F	PTU	18 mo	2 yr	−	−	NT	−	NT	−
6	34	F	PTU	2 wk	1 wk	−	+	NT	−	NT	+
7	30	F	PTU	2 mo	4 yr	NT	NT	NT	−	NT	+

NT, Not tested.
[a] Time from commencement of therapy to onset of agranulocytosis.
[b] SI more than upper limit of normal (mean + 2 SD for normal subjects).
[c] Determined using a modified Coombs' test.

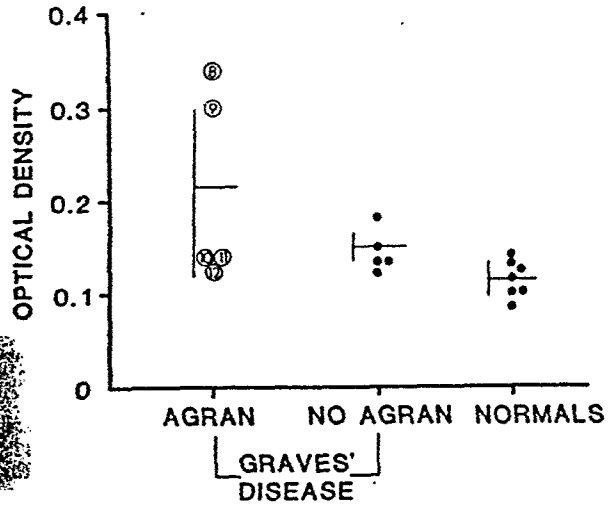

FIG. 2. Antibodies against normal human neutrophils in: 1) patients with PTU-induced agranulocytosis (AGRAN) determined using an ELISA. Results are expressed as od at 410 nm. 2) Patients currently being treated with PTU without reaction and 3) normal subjects. *Horizontal bars*, mean values (±SE). O, for patients with agranulocytosis; *numbers* represent individuals with agranulocytosis and correspond to those in Table 1.

drug reactions are usually associated with immunoglobulin E type hypersensitivity occurring in atopic individuals, there have been several reports of *in vitro* peripheral blood lymphocyte transformation in response to drugs in patients with allergies to the drugs (13–16). Moreover immunoglobulin G responses to drugs are not unexpected. The demonstration in this study of significant *in vitro* peripheral blood lymphocyte transformation in response to antithyroid drugs in 5 of 6 patients with Graves' disease who developed agranulocytosis while being treated with PTU (or CARB in one patient) and of circulating antibodies against neutrophils in 7 of 12 such patients suggests that immune reactions play a role in the depression of granulopoiesis. Because tests were always negative in patients currently being treated with antithyroid drugs it appears unlikely that immune responses to antithyroid drugs is a standard consequence of antithyroid drug treatment. Because there is bone-marrow depression in these patients the immunologically mediated damage presumably affects the stem cells rather than mature, circulating neutrophils. In view of the extensive cross-reaction demonstrated it is likely that the thioamide group, which is common to the 3 antithyroid drugs, is either the antigen or becomes antigenic by forming a complex with a neutrophil protein. Such a complex may act as a nonspecific stimulant (adjuvant) of the immune system (17) with bystander neutrophil damage.

Although, using the Coombs test, antibodies against neutrophils were detected only in the presence of antithyroid drug, this was not the case for those detected using an ELISA in which tests were equally positive with or without the addition of antithyroid drugs. The reason for this apparent discrepancy is unknown although likely to reflect either the different sensitivity of the tests or the existence of separate classes of antibodies, *i.e.* 1) those causing agglutination (detected in the Coombs test) which may react with a neutrophil-drug complex, 2) those which bind to but do not agglutinate (detected in an ELISA), and which may be directed against a neutrophil protein independent of the antithyroid drug, and 3) those which react with antithyroid drug alone. Of the two tests the ELISA is certainly the more reliable and specific, being a well standardized and widely used technique. Neutrophil agglutination is, on the other hand, much more prone to nonspecific influences and is difficult to standardize. The finding of antibodies against antithyroid drugs therefore needs to be confirmed, *e.g.* by binding of [^{35}S]PTU by patient immunoglobulin.

Although *in vitro* PBL transformation in response to antigen is generally thought to represent a cell-mediated (T cell) reaction, antibody producing (B) cells may also transform under these conditions. Hence, while positive lymphocyte transformation to antithyroid drugs suggests hypersensitivity, it gives little indication of the underlying immunological mechanism of the agranulocytosis. Thus, the agranulocytosis may be due to cytotoxic antibodies, the development of agranulocytosis depending on the chance formation of antibodies of high affinity, or to cell-mediated immune reactions involving lymphokines. Antibody-dependent killer cell-mediated cytotoxicity against sensitized neutrophils may also be a mechanism for antithyroid drug-associated agranulocytosis, a possibility that has not been studied.

Graves' disease is associated with both humoral and cell-mediated immunological reactions against several tissues including thyroid (18–21), orbital tissue (22–25), and lacrimal gland (26). Although the underlying mechanism is unknown, the resulting abnormality is a defect in lymphocyte surveillance (27–31) which may be antigen specific (32, 33). It seems likely that immunoreactivity to antithyroid drugs is another expression of the abnormality of the immune system in these patients. Thus both drug allergies and autoimmune disorders occur with increased frequency in patients with immunodeficiency (34–36). In this respect it may be significant that the patient with negative lymphocyte transformation and antibody tests (patient 1) had the greatest delay, 1½ yr, between the commencement of treatment and the development of agranulocytosis.

It is a common clinical experience that patients who develop sensitivity responses to a single antithyroid drug can usually be changed to another of these drugs without difficulty. This tolerance for alternative drugs may merely reflect differences in affinities of antibodies for

one or other of the drugs. The extensive immunological cross-reactivity demonstrated in this study and, particularly, the possibility of occult sensitivity phenomena as a result of this interaction suggests that this practice should be avoided.

Acknowledgments

The authors wish to thank Dr. C. Stewart and members of the Pathology Department, Repatriation General Hospital, Greenslopes, Australia, for performance of the antibody tests.

References

1. McCormick RV 1950 Periarteritis occurring during propylthiouracil therapy. J Am Med Assoc 144:1453
2. Wing ES, Asper SP 1952 Observations on the use of propylthiouracil in hyperthyroidism. Bull John Hopkins Hosp 90:201
3. Walzer RA, Einbinder J 1963 Immunoleukopenia as an aspect of hypersensitivity to propylthiouracil. J Am Med Assoc 184:743
4. Best MM, Duncan CH 1964 A lupus-like syndrome following propylthiouracil administration. J Ky Med Assoc 62:47
5. Amrhein JA, Kenny FM, Ross D 1970 Granulocytopenia, lupus-like syndrome, and other complications of propylthiouracil therapy. J Pediatr 76:54
6. Cooper DS, Goldminz D, Levin AA, Ladenson PW, Daniels GH, Molitch ME, Ridgway EC 1983 Agranulocytosis associated with antithyroid drugs: effects of patients age and drug dose. Ann Int Med 98:26
7. Laberi M, Sterling F, Utiger RD 1975 Reduction in extrathyroidal triiodothyronine production by propylthiouracil in man. J Clin Invest 55:218
8. Geffner DL, Azukizawa M, Hershman JM 1975 Propylthiouracil blocks extrathyroidal conversion of thyroxine to triiodothyronine and augments thyrotropin secretion in man. J Clin Invest 55:224
9. Wall JR, Lee Manwar G, Greenwood D, Walters BA 1976 *In vitro* suppression of lectin-induced ^{3}H-thymidine incorporation into DNA of peripheral blood lymphocytes after the addition of propylthiouracil. J Clin Endocrinol Metab 43:1406
10. Hallengren B, Forsgren A, Melander A 1980 Immunologic effects of methimazole and propylthiouracil *in vitro*. In: Stockigt JR, Nagataki S (eds) Thyroid Research Proc. VIII Int. Thyroid Congr. Australian Academy of Science, p 625 Sydney, Australia
11. McGregor AM, Peterson MM, McLachlan SM, Rooke P, Rees S, Smith B, Hall R 1980 Carbimazole and the autoimmune response in Graves' disease. N Engl J Med 303:302
12. Böyum A 1968 Separation of leukocytes from blood and bone marrow. Scand J Clin Lab Invest [Suppl 97] 21:9
13. Hirschhorn K, Bach F, Kolodny RL Firschein IL, Hashem N 1963 Immune responses and mitosis of human peripheral blood lymphocytes *in vitro*. Science 142:1185
14. Pearman G, Lycette RR, Fitzgerald PH 1963 Tuberculin-induced mitosis in peripheral blood leukocytes. Lancet 1:637
15. Holland P, Mauer AM 1964 Drug-induced *in vitro* stimulation of peripheral lymphocytes. Lancet 1:1368
16. Reidenberg MM, Caccese RW 1975 Lymphocyte transformation tests and suspected drug allergies. J Lab Clin Med 86:997
17. Schoen RT, Trentham DE 1981 drug-induced lupus: an adjuvant disease? Am J Med 1:5
18. Doniach D, Roitt IM 1962 Autoimmunity and disease. What's New? 229:22
19. Volpé R 1972 The Immunological basis of Graves' disease. N Engl J Med 287:463
20. Volpé R, Farid NR, Con Westarp C, Row VV 1974 The pathogenesis of Graves' disease and Hashimoto's thyroiditis. Clin Endocrinol (Oxford) 3:239
21. Wall JR, Trewin A, Joyner DM 1980 Peripheral blood lymphocyte transformation in response to human thyroid fractions in patients with Graves' hyperthyroidism and ophthalmopathy. Acta Endocrinol (Copenh) 93:419
22. Mahieu P, Winand R 1972 Demonstration of delayed hypersensitivity to retrobulbar and thyroid tissues in human exophthalmos. J Clin Endocrinol Metab 34:1090
23. Lamki L, Row VV, Volpé R 1973 Cell-mediated immunity in Graves' disease and in Hashimoto's thyroiditis as shown by the demonstration of migration inhibition factor (MIF). J Clin Endocrinol Metab 36:358
24. Munro RE, Lamki L, Row VV, Volpé R 1973 Cell-mediated immunity in the exophthalmos of Graves' disease as demonstrated by the migration inhibition factor (MIF) test. J Clin Endocrinol Metab 37:286
25. Wall JR, Walters BAJ, Grant C 1979 Leukocyte adherence inhibition in response to human orbital and lacrimal extracts in patients with Graves' ophthalmopathy. J Endocrinol Invest 2:375
26. Wall JR, Trewin A, Fang SL, Ingbar SH, Braverman LE 1978 Studies of immunoreactivity to human lacrimal gland fractions in patients with ophthalmic Graves' disease. J Endocrinol Invest 1:253
27. Playfair JHL 1975 Autoimmunity in Endocrine Disease. J Clin Endocrinol Metab 4:229
28. Allison AC, Denman AM 1976 Self-tolerance and autoimmunity. Br Med Bull 32:124
29. Asherson GL, Zembala M 1976 Suppressor T-cells in cell-mediated immunity. Br Med Bull 32:158
30. Wall JR, Fang SL, Ingbar SH, Braverman LE 1976 Lymphocyte transformation in response to human thyroid extract in patients with subacute thyroiditis. J Clin Endocrinol Metab 43:587
31. Kidd A, Okita N, Row VV, Volpé R 1980 Immunological aspects of Graves' and Hashimoto's disease. Metabolism 29:80
32. How J, Topliss DJ, Lewis M, Row VV, Volpé R 1981 Graves disease (GD) may be characterized by an inherited defect in suppressor T-lymphocyte function. Ann Endocrinol 42:28A
33. Okita N, Row VV, Volpé R 1981 Suppressor T-lymphocyte deficiency in Graves' disease and Hashimoto's thyroiditis. J Clin Endocrinol Metab 52:528
34. Gatti RA, Good RA 1980 Immunological deficiency diseases. Med Clin N Am 54:281
35. Fudenberg HH 1971 Are autoimmune diseases immunologic deficiency states? In: Good RA, Fisher DW (eds) Immunobiology: Current Knowledge of Basic Concepts in Immunology and Their Clinical Applications. Sinauer Associates, Stamford, CT, p 175
36. Osaba DM 1972 Thymic function, immunologic deficiency and autoimmunity. Med Clin N Am 56:319